CAR-T cell Therapy in B-cell Lymphoma

By

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A thesis submitted to the Department of Pharmacy in partial fulfillment of the requirements for the degree of
Bachelor of Pharmacy (Hons)

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Declaration

It is hereby declared that

1. The thesis submitted is my/our own original work while completing degree at Brac University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. I have acknowledged all main sources of help.

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Approval

The project titled "CAR-T cell Therapy in B-cell Lymphoma" submitted by Asef Faruk (15146002) of Spring, 2015 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy (Hons.) on August, 2019

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Ethics Statement

This study does not involve any human or animal trial

Abstract

B-cell lymphoma continues to be difficult to treat, with insignificant advances seen in more than two decades despite improvements in initial treatment strategies across its subtypes. Developing new targeted therapies which have acceptable safety and toxicity profiles is critical for better survival chances of the patients. Immunotherapy provides promising possibilities for patients with different B-cell lymphomas. CAR-T cell therapy has emerged as a new generation of powerful immunotherapy that has demonstrated high response in clinical trial for different B-cell lymphoma patients. The process involves adoptive transfer of T cells engineered ex vivo to be redirected to cancer cells. Recent clinical trials have provided promising safety and efficacy data for different B-cell lymphomas which have garnered enthusiasm in the scientific community. This review discusses the design of CAR-T cell therapy, its clinical trials and safety and efficacy strategies for successful clinical and commercial translation.

Keywords: Cancer; CAR-T cell; B-cell lymphoma; Immunotherapy.

Dedication

Dedicated to all the cancer patients

Acknowledgement

I would like begin by thanking the Almighty Allah, our creator, the source of our life and strength, our knowledge and wisdom, for the blessings and mercy. All praises to the Almighty Allah and I would like to express my gratitude for blessing me with immense patience, strength, gratefulness and assistance when necessary to complete this project.

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List of Acronyms

ACT	Adoptive cell therapy
ALL	Acute lymphocytic, lymphatic or lymphoblastic leukemia
BCR	B cell receptor
CAR	Chimeric Antigen Receptor
CLL	Chronic Lymphatic, or lymphoblastic leukemia
CTLA 4	Cytotoxic T-lymphocyte-associated protein 4
DLBCL	Diffuse large B-cell lymphoma
MALT	Mucosa associated lymphoid tissue
MHC	Major histocompatibility complex
PD-L1	Programmed death-ligand 1
PMBCL	Primary mediastinal B-cell lymphoma

Chapter 1

Introduction

1.1 Cancer

Cancer can be described as a generalization encompassing a plethora of interconnected diseases (Hassanpour & Dehghani, 2017; “What Is Cancer?,” 2019). It is a disease resulting from genetic mutations and epigenetic changes in malignant cells (Hemminki & Hemminki, 2005). It is featured by excessive cell growth or cell proliferation ignoring the control signals of cell division, thus spread all over the body from the site of origin. Cancer can emerge from unregulated proliferation of any of the various kinds of cells in body, thus, more than 100 distinct types of cancers have been classified. The discerning characteristics of each type of cancer depends on the primary tissue or site of origin of carcinogenesis (Hejmadi, 2010; Pecorino, 2012). Any uncontrolled proliferation of cells is called a tumor, which sequentially escalates into this disease. The most significant element of cancer pathology is the discernment between the two types of tumor, benign and malignant tumor. A harmless or benign tumor is constricted to its primary site and does not spread or invade into its surroundings. However, a malignant tumor is able to spread throughout the system via the circulatory or lymphatic system. The incident of a malignant tumor spreading to its surroundings and throughout the body is termed as “metastasis”, and it is the ability to metastasize which makes cancer so pernicious (Cooper, 2000). Genetic abnormalities contribute 7% to the pathogenesis of cancer and the remaining of 93% is caused by environmental factors (Nooshinfar et al., 2017).

1.1.1 Types of cancer

Types of cancer can be separated into two sub groups depending on their type of tissue or their origin site.

Classification based on site of origin

Cancer can be termed as being of different kinds, including brain cancer, breast cancer, liver cancer, prostate cancer etc., depending on their site of origin.

Classification based on tissue types

Cancers can be divided into six primary categories.

Carcinoma

Carcinoma, the most common subgroup of cancer, is initiated from cells of the epithelial cell layer that forms our body and organs' external lining or internal lining. As epithelial cells are most abundant in the body, carcinomas account for 85% of all cases of cancer (Pecorino, 2012). Carcinomas mostly affect secretory organs or glands, such as prostate, lungs, bladder, breasts. Carcinomas have specific names based on various types of epithelial cells.

Glandular tissues are tissues with these types of epithelial cells. Most colon, prostate and breast cancers are named to be adenocarcinomas. Carcinoma of basal cell starts in the epidermis ' lower layer, which is the outermost skin layer. Squamous cells are the site for squamous cell carcinoma. Also, many other organs are lined by squamous cells, including the intestines, stomach, lungs, kidneys and bladder. Another name of squamous cell carcinoma is epidermoid carcinoma. Transitional cell carcinoma forms in the transitional epithelium or urothelium which are types of epithelial tissue. This tissue consists of several layers of epithelial cells which grow smaller and larger and is observed in bladder linings, ureters, and renal pelvis and also other organs.

Sarcoma

Sarcomas occur in body tissues that are connective and supportive. Soft tissue sarcoma is called osteosarcoma or bone cancer and is seen commonly. Following are the examples of other places affected by sarcoma:

- Cartilage (chondrosarcoma)
- Smooth muscles (leiomyosarcoma)
- Skeletal muscles (rhabdomyosarcoma)
- Fibrosarcoma
- Blood vessels
- Liposarcoma
- Glioma or astrocytoma
- Myxosarcoma
- Mesenchymous or mixed mesodermal tumor

Leukemia

Grouped into blood cancers, leukemia tends to affect the bone marrow that produces blood cells. When cancerous, rather than developing solid tumors, the bone marrow generates excess amounts of immature white blood cells that create difficulties in supplying oxygen to tissues and also in case of fighting infections. Leukemia types include:

- Acute myelocytic leukemia (AML)
- Leukemia in adults: Chronic myelocytic leukemia (CML)
- Acute lymphocytic, lymphatic or lymphoblastic leukemia (ALL)
- Leukemia observed in the elderlies: lymphocytic, Chronic Lymphatic, or lymphoblastic leukemia (CLL)

- Predominant red blood cell production: Polycythemia vera or erythremia

Lymphoma

Lymphoma can be defined as a subgroup of cancer that targets human immune system's T or B lymphocytes. It is defined by irregular lymphocyte formation in the lymph system and other body regions. There may be two types of lymphomas – the lymphoma of Hodgkin and the lymphoma of Non-Hodgkin. There is a hallmark existence of Reed-Sternberg cells in the cell's tissue models in Hodgkin lymphoma unlike in the other type.

Melanoma

Melanoma originates in a cell called melanocytes. Melanocytes which is responsible for producing melanin. Melanomas are mostly formed on skin surface, but also occurs in other tissues which are pigmented like eyes (Mandal, 2012; "What Is Cancer?," 2019).

1.1.2 Prevalence

With this growing number of incidences, cancer prevention is one of the key health issues of the 21st century. 17 million novel cases of cancer were reported (all cancers combined excluding non-melanoma skin cancer) around the globe in the year 2018. Of these cases 8.8 million patients (52%) were males and 8.2 million (48%) patients were females, forming a ratio of 10:9.3 (Bray et al., 2018). According to a study done in 2016, countries with high income have the maximum incidence rate for different subgroups of cancer. In many high-income nations, the rate of cancer deaths is declining while in low- or middle-income countries they are increasing. Countries that fall under lower and middle income bracket have the highest levels of stomach, liver, esophagus and cervix cancer (Torre, Siegel, Ward, & Jemal, 2016). Prostate cancer is a highly diagnosed cancer amongst men in 114 countries. Lung cancer is the highest diagnosed cancer in men in Eastern Europe. Among men, lung, liver, esophagus, leukemia, prostate, stomach, and non-Hodgkin lymphoma are the leading

cancers in Africa, while in Asia, lip, oral cavity, lung, liver, colorectal, prostate and stomach cancers take the lead. Breast cancer seems to be the most common amongst females in North America, Europe, and Oceania. In Latin America and the Caribbean, Africa and most of Asia, in women, breast and cervical cancers are most commonly diagnosed (World Health Organization International Agency for Research on Cancer, 2018). Lung, female breast, bowel and prostate are observed to be the four most highly occurring cancers that occur worldwide and account for more than four out of ten of all diagnosed cancers worldwide. It is believed that by 2040 ,27.5 million new cancer cases will occur every year. In 2018, there were 9.6 million deaths from cancer worldwide, according to data obtained. Lung, liver, stomach and intestine cancers are known to be the four most common causes of cancer death since 1975 (Cancer Research UK, 2014).

1.1.3 Mechanism of carcinogenesis

Cancer refers to the uncontrolled proliferation of cells because of mutations inside genes regulating cell growth and division, which may take place due to complex interactions between environmental and endogenous factors. Cancer has a particular coalition of genetic changes from individual to individual, cell to cell and additional changes occur with its progress (Arley & Eker, 2013; “What Is Cancer?,” 2019). The genetic variation in case of tumor suppressor genes, proto-oncogenes and DNA repair genes mainly contribute to cancer, also known as “drivers” of cancer.

Normal growth of cells and their division are dependent on proto-oncogenes and tumor suppressor genes. However, cancer-causing genes or oncogenes may emerge due to alterations in these genes, allowing cells to proliferate and survive uncontrollably (Shtivelman, Lifshitz, Gale, & Canaani, 1985).

Genes which are called DNA repair genes are responsible for fixing DNA which are damaged. Cells that contain mutations in DNA repair genes have the habit of developing more mutations genes of other origins. All these mutations are responsible for converting the cells to cancerous types (Wei, Li, & Chen, 2012).

Cancer progresses in 3 consecutive stages of initiation, promotion and progression.

Initiation

Initiators act directly or indirectly by producing electrophilic species. These species intermingle with and alter DNA structures and impairs the sequence of DNA. Initiation is thought to create a lesion that perseveres over an extended length of time (Van Duuren, Sivak, Katz, Seidman, & Melchionne, 1975). In an experimental study, it was shown that mouse skin which was treated at a time which is more than a period of time that was one year ago that is phorbol esters was still very vulnerable to induction of tumors. Thus, it can be said that initiation is a step which is irreversible. In addition of the above-mentioned phenomenon, it was also proved that repeated doses of initiators result in additive number of tumor formation (BOUTWELL, 1964).

Promotion

Promoter causes a carcinogenic response when multiple doses of it are applied following a single, sub carcinogenic dose of an initiator. Promoters by itself is non-carcinogenic. This time-dependent sequence of administration of promoter and initiators can only be shown in the laboratory. It is difficult to identify such timely procedures in humans as they take concurrent environmental exposures from a variety of substances that are chemical in nature. Few promoters have shown an initiating ability which is weak at elevated doses. Promoters are known not to form electrophilic species like initiators. Effects which are caused by a promoter that is characterized as first-stage are believed to be reversible; i.e., if the exposure

of a promoter is stopped, it does not produce a carcinogenic response. When administered, second-stage promoter produces irreversible effects.

Compounds which can simultaneously act on the same tissue as an initiator and a promoter is known as a complete (whole) carcinogen. Most initiators usually are complete carcinogens.

Progression

The time required for forming a tumor which is benign in nature to get malignant is dubbed as progression. The process for the transformation requires several stages. The stages include oncogene activation (Weinberg, 1985), chromosome aberration (Weinstein et al., 1984), interaction between tumor cells and host defenses, and various selection processes ("Chemical carcinogens: a review of the science and its associated principles. U.S. Interagency Staff Group on Carcinogens.," 1986). In progression stage, tumors might get severe in terms of malignancy and heterogeneity and this is a dynamic process (Weinstein et al., 1984).

1.2 Cancer Immunotherapy

Immunotherapy facilitates our immune system to overcome immune escape characteristics of malignant cells from immune surveillance and flags the tumor cells and bolsters our immune system by augmenting substances for immune elimination (Gregory L. Beatty & Gladney, 2015; Oiseth & Aziz, 2017). Thus, cancer immunotherapy has emerged as one of the most beneficial ways of dealing with cancer. Various types of immunotherapies are currently employed to cure cancer using different components of our immune system in disparate ways.

Adoptive cell therapy (ACT) is a subgroup of immunotherapy that incorporates isolation and *in-vitro* expansion of infiltrative and tumor-specific T-cells. After that the process was to infuse them back into the body (Guillerey, Huntington, & Smyth, 2016). Different forms of

ACT include culturing and expanding tumor-infiltrating lymphocytes taken from tumors; or modifying T-cells to have receptors that identify and attack tumor cells. The latter is dubbed as chimeric antigen receptor T-cell (Oiseth & Aziz, 2017).

The magnitude and quality of immune response are reliant on the antigens being recognized by T-cell receptors and an equilibrium between the co-stimulatory signals and inhibitory signals, known as immune checkpoints. Tumor cells can upregulate the expression of inhibitory signals as a part of their protective mechanism. Such inhibitory molecules include CTLA4, also known as CD152 and programmed cell death protein 1 (PD1) (Pardoll, 2012). Immune checkpoint inhibitors are used to inhibit expression of inhibitory signals and reinforce the immune system. Monoclonal antibodies that target CTLA4 and PD1 are employed to inhibit the negative blocking of T-cells. Nivolumab and Ipilimumab are such antibodies (Postow et al., 2015).

Oncolytic viruses are being used which have been altered to lack virulence against normal cells and lyse the malignant cells, which are further attacked due to a release of antigens following the lysis (Choi, O'Leary, Fong, & Chen, 2016). T-VEC, which is a herpes simplex-1 virus is known to be sanctioned by the FDA for treating advanced melanoma (Oiseth & Aziz, 2017).

Another type of immunotherapy involves recombinant cytokines such as IL-2 (Proleukin), which the FDA has approved for treating melanoma and renal cell cancer. Interferon α is another such agent (Nisbet, 2016).

The advancement in cancer immunotherapy has shifted the focus to treating biological traits of cancer cells from treating disease site. Immunotherapy will always provide innate advantages over other therapies due to its ability to memorize and identify malignant cells. The future hurdles in the field of immunotherapy will be to detect the cause of fluctuation in

the success rate of the treatment and to modify the immune system in patients lacking immune response to cancer cells, even when the tumor microenvironment is completely barren of infiltrating T-cells (Hegde, Karanikas, & Evers, 2016). A recent study has used the CRISPR technique to detect gene mutations in malignant cells in patients who have failed immunotherapy (Patel et al., 2017).

Chapter 2

B-cell lymphoma

2.1 Incidence

NHL is comparatively more usual in males than females in a study conducted on Caucasian heritage people. Based on reported 2012-2016 cases, the number of new non-Hodgkin lymphoma cases was 19.6 per 100,000 people per year (National Cancer Institute, 2018).

Diffuse B-cell lymphoma develops inside lymph system from B-cells. DLBCL counts up to thirty to forty percent of all non-Hodgkin lymphoma cases (Leukaemia Foundation, 2019) . Global epidemiological data on DLBCL is lacking, but according to a study done in 2015, the projected occurrence is seven per 100,000 in United States (Li, Wang, Wang, Yi, & Ma, 2015). The disease is more prominent in elder patients than in younger ones (Cairo & Perkins, 2013). According to NIH, the mean diagnosis age is 66.

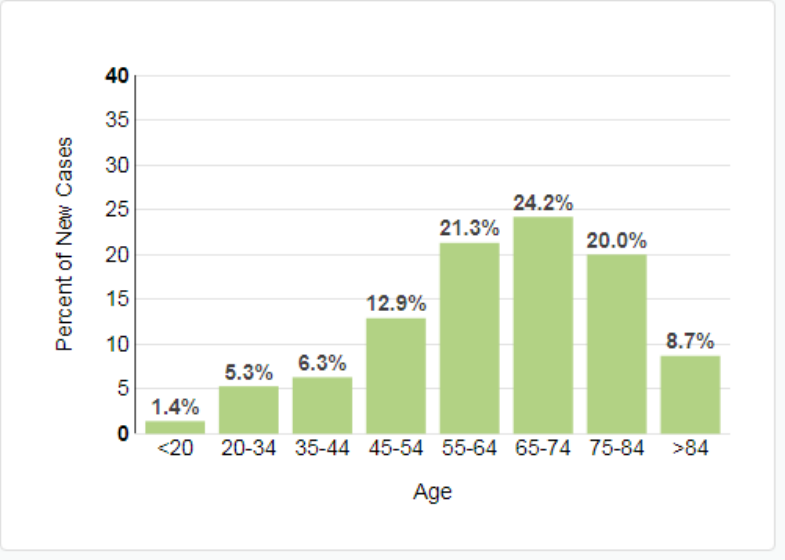

Figure 1- Percent of DLBCL New Cases by Age Group (National Cancer Institute, 2018)

Based on a study done between, 2012-2016, the count of new cases of DLBCL was 5.6 per hundred thousand males and females per year (U.S. National Institute Of Health, 2015). It is generally seen that if the cancer is diagnosed early to increases the chances of survival chances as well as treatment opportunities. After being diagnosed, in case of DLBCL, 24.8 percent usually receive diagnosis at stage I. The survival percentage for stage I DLBCL is 73.1%., for stage II its 72.1 percent, for stage III 63.2 percent and for stage IV 51.1 percent (National Cancer Institute, 2018). One variant, Primary gastric lymphoma has an incidence in Western countries of 1 per 100,000 of the population. It constitutes 60% of all gastrointestinal lymphomas, and 20% – 30% of extranodal NHLs (Pohl, 2013).

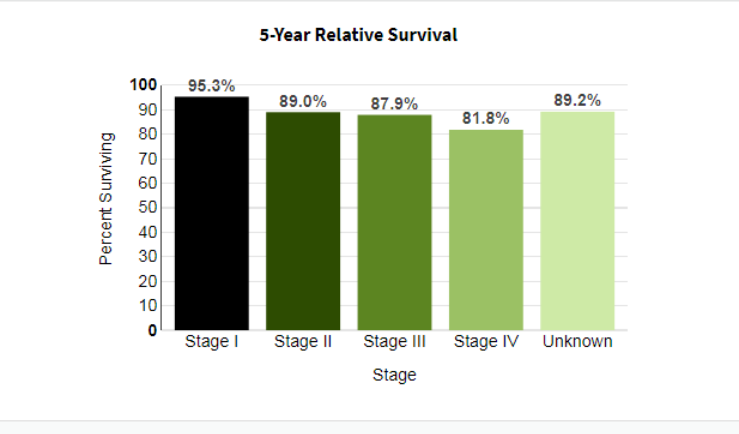

Figure 2-5-Year Relative Survival by Stage at Diagnosis: Follicular Lymphoma (National Cancer Institute - Surveillance, Epidemiology, 2019)

In the United States, 74,200 cases of NHL are predicted to occur in 2019 and approximately 20% are predicted to be follicular lymphoma (Communication, 1997; Luminari, Bellei, Biasoli, & Federico, 2012). According to the portal of rare disease, the prevalence of this disease is a person among every 30,000 people (McNamara et al., 2012). Based on studies done in different age groups it is seen that the age group 55-74 comprises of 53.9 percent of the diseased patients, followed by the age group 45-54 that make up 17.7 percent of the diseased patients (National Cancer Institute - Surveillance, Epidemiology, 2019).

CLL or of chronic lymphocytic leukemia accounts for about one-quarter of the new leukemia cases annually. There is a 0.57% of risk involved for an average person to get CLL (American Cancer Society, 2018). CLL commonly occurring leukemia in case of adult who are older than 19 and accounts for 37 percent cases. In USA, in 2019, it is predicted that 20,720 people will receive a diagnosis of CLL across all age categories among which 2,880 men and 7,840 women are expected to be women. CLL is widely seen in older people and 9 percent the patients suffering from CLL are 50 or older. CLL is rare in individuals under 40 years of age and very rare in kids (cancer.net, 2016). According to studies, the percent of death from each age group is as followed:

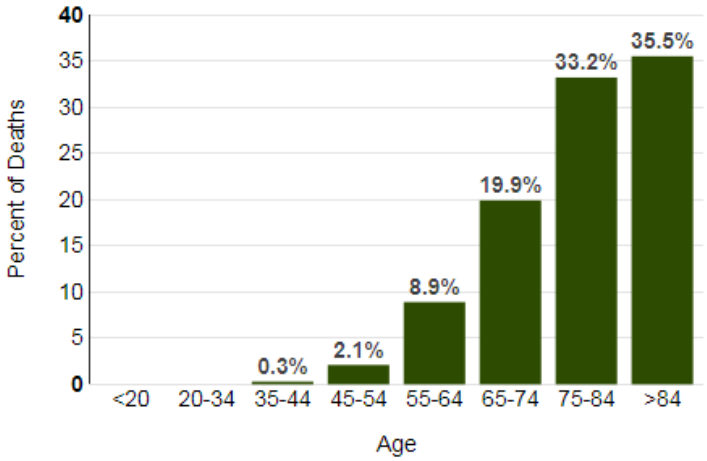

Figure 3- Percent of Deaths by Age Group: Chronic Lymphocytic Leukemia (National Cancer Institute Surveillance, 2018)

Marginal zone B-cell lymphoma constitute about 5% of observed non-Hodgkin lymphoma cases that are identified in patients per year. The rate is estimated at about 1/313,000 (“Marginal-zone lymphoma | Startoncology,” 2019; ORPHANET, 2010a).

Marginal zone lymphoma of MALT is 7% of all non-Hodgkin lymphomas (“Marginal Zone B-cell Lymphoma: Definition, Epidemiology, Etiology,” 2014). The rate of incidence of marginal zone lymphoma is nearly equivalent among males and females with the exception

of older aged people, when incidence of the diseases is more common among males compared to females. The ratio stands as male: female ratio = 1.27 (Khalil et al., 2014). Mostly all gastric MALT lymphoma patients are disease-ridden with Helicobacter pylori (Parsonnet et al., 1994) and with decline of H pylori infection, the rate of incidence of MALT is also falling (Blaser, 2002; D. Zhang et al., 2009).

Mantle cell lymphoma (MCL) affects three percent to six percent of patients with NHL and is a rare subgroup of B-cell NHL (Fu et al., 2017). It amounts to 2-10% of all lymphoma subtypes. The projected number of lymphomas is 1 in every 25,000 people. Mantle cell lymphoma is known to affect people who are between the ages 35-85 years. The quotient of male to female is 1:4 meaning that men are more susceptible to the disease than females. Only thirty percent of the total patients have a complete response to present therapies (ORPHANET, 2010b).

2.2 Pathogenesis

B cells start developing within the bone marrow and stops after successful reorganization of immunoglobulin (Ig) heavy chains and light chains and armed with a functioning antigen receptor on the surface by a B-cell precursor. Cells having functioning receptor B-cell's surface on the bone marrow after differentiating into mature B cells. However, cells that fail to express a functioning B-cell receptor, go through apoptosis (Rajewsky, 1996). Mature naïve B cells can play their role in immune responses after an antigen binds to the BCR and activates the B cell. Antigen-activated B cells go through clonal expansion, in case of T-cell-dependent immune responses and this expansion transpire in structures known as germinal centers (GCs). GCs, through somatic hypermutation and class switching, Ig genes get altered. Distinct structures of the BCR and expression of differentiation markers help to characterize different levels of B cell maturation and differentiation. These processes of development and

differentiation occurs inside certain structures and the source of various B-cell lymphomas are identified by analysis of these traits (Ralf Küppers, Klein, Hansmann, & Rajewsky, 2002; Stevenson et al., 2001). It has been observed that, cancerous B-cells seem 'frozen' at a distinct differentiation step and the reasoning for such differentiation of B-cell lymphomas is found on this observation (Greaves, 1986; Ralf Küppers et al., 2002; Shaffer, Rosenwald, & Staudt, 2002). From these studies, one of the major observations is that 'germinal center' (GC) and post GC B-cells are the origin of most types of B-cell lymphomas (Ralf Küppers et al., 2002; Stevenson et al., 2001).

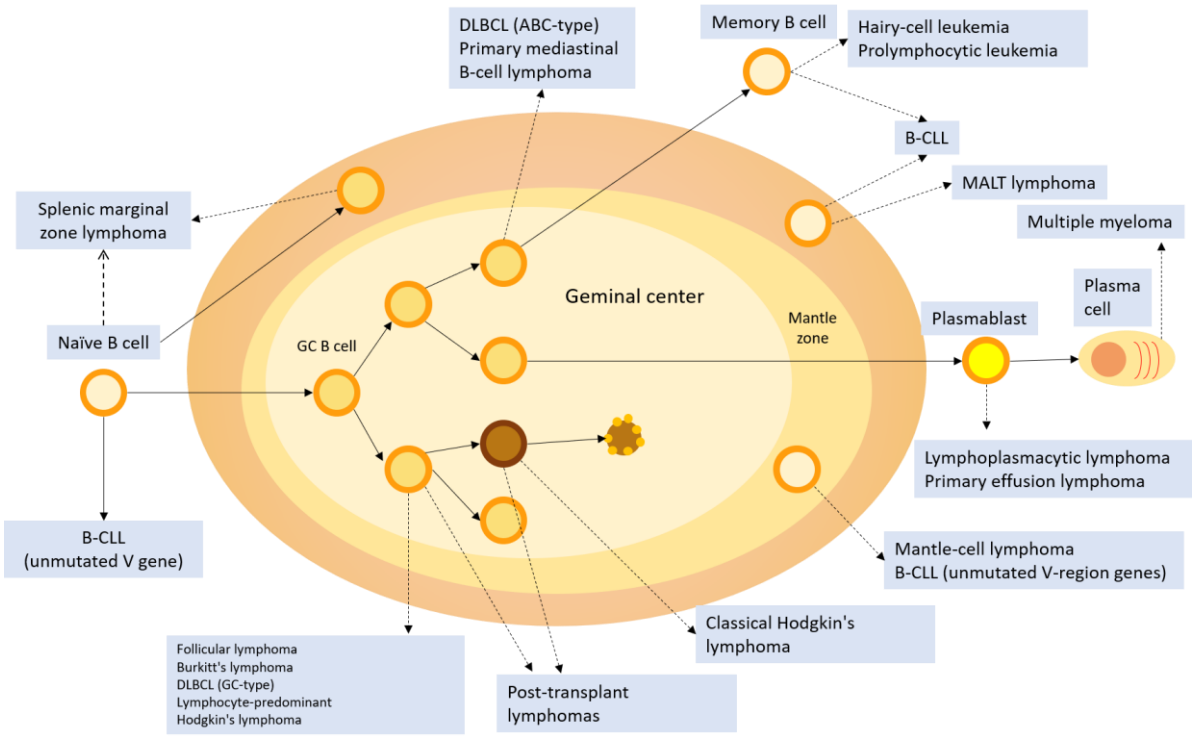

Figure 4-Cellular origin of B-cell lymphomas (Ralf Küppers, 2005)

After being activated by antigen-receptor binding followed by stimulation from T-cells, mature naïve B-cells get steered in primary B-cell follicles situated in secondary lymphoid organs and form germinal centers (MacLennan, 1994). Proliferating GC B-cells replace the naïve IgM+IgD+ B cells constituting the primary B-cell follicle and displace them outside the GC. The displaced cells create a mantle zone surrounding the GC. The GC comprises of a dark zone which houses the proliferating B cells and a light zone which houses the resting

GC B cells (MacLennan, 1994). Somatic hypermutation gets activated in the proliferating B cells leading to the introduction of mutations to the rearranged Ig variable region genes at a high rate (R Küppers, Zhao, Hansmann, & Rajewsky, 1993). These mutations might cause cells to go through apoptosis if antigen affinity of BCRs decrease. However, GC B cells get positively selected following an increased BCR affinity for antigen due to the mutations. The process of selection presumably mainly transpires within the light zone. Here, the GC B-cells stay in close proximity with CD4+ T cells and follicular dendritic cells (FDCs) (R Küppers et al., 1993).

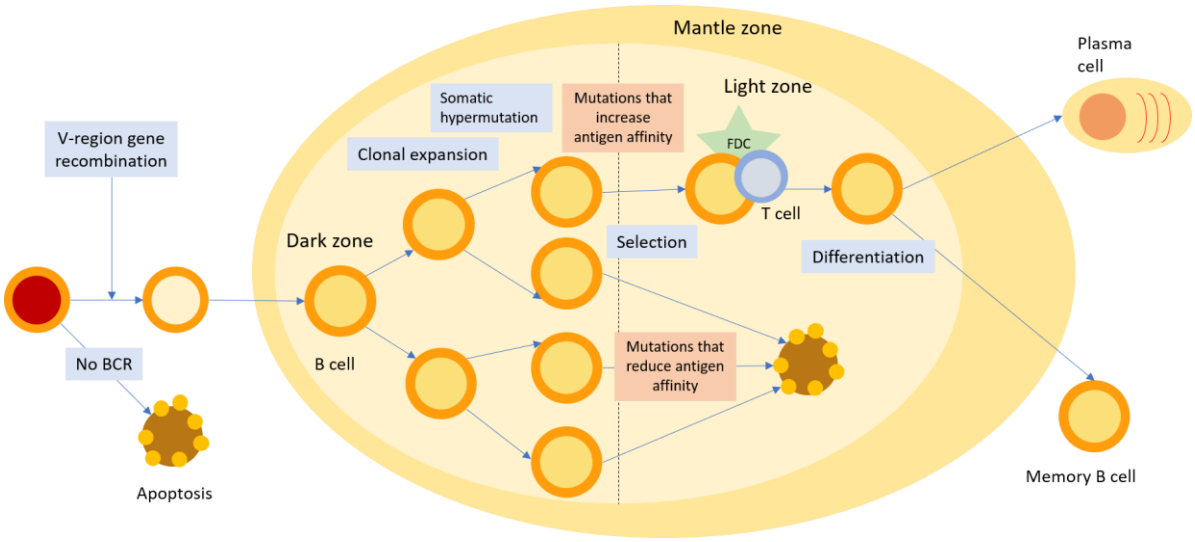

Figure 5-Germinal-center reaction of B-cell differentiation (Ralf Küppers, 2005)

The proposed normal B-cell counterpart is assigned to B-cell lymphomas. Many lymphomas are obtained from germ line r(GC) B-cells or B-cells passing through the GC, suggesting their function in B-cell lymphoma's pathophysiology. As the figure shows, the GC is encircled by a mantle zone of naive B cells, many of them express the CD5 marker — these may consist of a distinguishable subgroup of B-cells. The marginal zone is a B-cell-rich region located in the spleen between the T-cell area (an analogous area is found in Peyer's patches, but not usually in lymph nodes) and B-cell follicles. The source of marginal B cells is a subject of dispute and is likely to include post-GC memory B cells and naive B cells

which are involved in T-cell-independent immune responses. Splenic B-cell marginal-zone lymphomas consist of both follicular and marginal-zone B cells and sometimes even carry non mutated (V)-region genes. Therefore, these lymphomas could be obtained from naive B cells that are susceptible to marginal B-cell differentiation (Dogan & Isaacson, 2003). The source of B-cell chronic lymphocytic leukemia (B-CLL) cells has been debated. Approximately half of BCLL cases carry V-region gene mutations. Both B-CLL subgroups were suggested to be derived from either from CD5 + B cells, B memory cells or B marginal cells (Chiorazzi & Ferrarini, 2003). Profiling of gene expression identified two major subgroups of DLBCL, one with a similar profile to GC B cells (GC-type), and the other similar to in-vitro-activated B cells (ABC-type) It is believed that primary mediastinal B-cell lymphomas originate from thymus post-GC B cells. Molecular mechanisms that reconfigure the genes of immunoglobulin. Igs are expressed by B-cells and consist of regions of variable region (V) that interact with antigen and regions that are constant (C) and that mediate Igs effector functions. In order to produce a functional Ig, B cells always rearranges portions of DNA encoding the variable gene's heavy (H) and light chains. Firstly, three sequences of genes, VH, DH and JH, are merged to encode the H-chain variable region by a process called ' V(D)J recombination. Alternatively, two gene segments — VL and JL genes (encode each of the V regions of the κ- and λ-light chains. B-cell precursors perform rearrangements of DH – JH in H-chain genes (Rajewsky, 1996). About 50 functional sections of the VH gene, 27 segments of DH and 6 segments of JH are available in the germline to generate a diversified range of rearrangements of the VH gene. The variety is again enhanced by adding or removing nucleotides at the gene segment joining sites (Rajewsky, 1996). At the L-chain loci, the cells then carry out rearrangements. The Ig gene's V-region is ultimately linked to the Ig gene's C-region Ig gene b. The somatic hypermutation mechanism is triggered when B cells enter the core of the germ. This mechanism gives rise to the introduction in the rearranged V-

region of Ig genes of point mutations, deletions or duplications (R Küppers et al., 1993). Class changing tends to result in replacing the original H-chain C-region gene with another Ig gene.

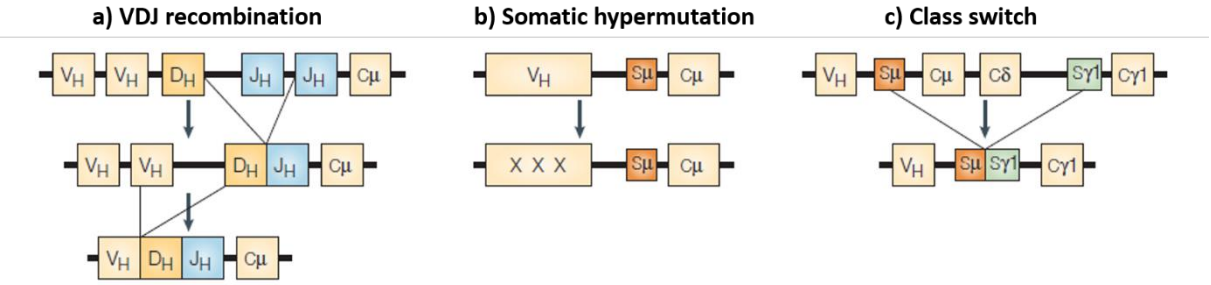

Figure 6-Alteration of immunoglobulin genes (Ralf Küppers, 2005)

2.3 Current therapies

DLBCL treatment

DLBCL, an unusually heterogeneous malady, shows substantial disparity in answer to therapy, clinical response and long-term treatment results (Alizadeh et al., 2000). Usual treatment procedures and DLBCL disease management tend to be different for localized disease (Ann Arbor stages I and II) and advanced-stage disease (stages III and IV), cyclophosphamide, hydroxydaunorubicin, vincristine, prednisolone (CHOP) chemotherapy which is given per 21 days (CHOP21) had continued to be the standard treatment for DLBCL until recently. The general long-term survival (OS) rate for CHOP21-treated patients is about 40 % (Fisher et al., 2002). In the year 1997, the FDA approved rituximab to be the very first sanctioned monoclonal antibody for the treating follicular lymphoma. After the approval, the immunotherapy was then used in treating DLBCL as well as other B-cell NHLs (Coiffier et al., 1998). Rituximab, an approved monoclonal antibody, is directed to work to inhibit CD20 protein. CD20 protein is largely seen on cellular surface of B cells. It exists for a multitude of lymphoma cells. Even though the process by which the drug works is not clearly understood, it is deduced that rituximab induces lysis of lymphoma cells by antibody-dependent cell

cytotoxicity, complement-mediated cytolysis in addition to direct induction of apoptosis. Additionally, rituximab works in a synergistic manner with chemotherapy (Cartron, Watier, Golay, & Solal-Celigny, 2004). Rituximab has proved to considerably improve disease results in DLBCL treatment. A clinical trial which was randomized (phase III study) done on a considerable number of DLBCL patients established enhanced OS in people given rituximab and CHOP (R-CHOP) therapy (Coiffier et al., 2007). Several different studies have proven that a joint rituximab and chemotherapy prolongs event-free survival (EFS) and OS in advanced age patients (Habermann et al., 2006; Sehn et al., 2005). Based on the clinical trials, R-CHOP therapy is currently thought to be the 'standard therapy' in DLBCL, specifically in case of elderly and in comparatively younger patients. However, this combination of therapy has failed to provide satisfactory results in patients of high-risk category in IPI classification (Kwak, 2012). The advantage of 3 cycles of CHOP combined with involved field radiotherapy (IFRT) for people who fall in the age range 60 years or younger and had showed no signs of adverse risk factors was confirmed by the British Columbia Cancer Agency (Shenkier et al., 2002).

Limited stage DLBCL can defined as the stage of DLBCL which can be limited to one irradiation field. 30%–40% of the total patients with DLBCL suffer from limited stage DLBCL. The primary treatment of limited stage DLBCL is joint modality therapy which consists of three cycles of abbreviated systemic chemotherapy, the recombinant anti-CD20 antibody rituximab, and involved field radiation therapy (RT). Another treatment is a regimen of six to eight cycle (full course) of (systemic chemotherapy plus rituximab without RT (Miller et al., 2002; Stephens et al., 2016). In people with non-bulky limited-stage DLBCL, treatment with abbreviated chemotherapy (R-CHOP × 3) plus involved-field radiotherapy (30–36 Gy) is the preferred modality of treatment compared to extended chemotherapy (R-CHOP × 6–8) unaccompanied. With respect to patients having persistent PET-positive illness

after chemotherapy higher doses of radiation (e.g., 45–50 Gy) may be a treatment option. The prognosis of disease for people with bulky (>10 cm) Stage II disease and advanced (Stage III or IV) disease are similar and thus their treatment regimen needs to be similar. Patients suffering from limited stage, bulky disease need to be treated with six cycles R-CHOP plus 30–40 Gy involved-field radiotherapy instead of only six or eight cycles of R-CHOP. In spite of this more aggressive approach, survival rates of patients suffering from bulky disease remain lower than for patients without bulky disease. This observation suggests that more clinical trials should be set up for this population (Miller, 2004).

60%–70% cases of DLBCL are cases of advanced stage large B- cell lymphoma. Treatment regimen in advanced cases require to be individualized for every patient on the basis of subtypes. Standard treatment for the higher percentage of patients with progressive disease is Chemoimmunotherapy. Based on numerous randomized study which were conducted before the dawn of rituximab containing regimen, R-CHOP is the preferred regimen compared to anthracycline based regimens (Bartlett et al., 2001; Gaynor et al., 2001; Magnus, Tomas, Anders, & Eva, 2008). In MabThera International Trial, patients under the age of 60 with DLBCL were randomly assigned and get treatment with six CHOP-like chemotherapy cycles given with or without rituximab every 21 days. People treated with R-CHOP showed pointedly higher remission rate, event-free, and OS (Pfreundschuh et al., 2006).

Primary mediastinal B-cell lymphoma (PMBCL) Treatment

Primary mediastinal B-cell lymphoma (PMBCL), a rare non-Hodgkin lymphoma subtype, mostly happens in adolescents and in young adults. Formerly PMBCL was thought to be a subgroup of diffuse large B-cell lymphoma (DLBCL) but WHO bow recognizes it as separate disease due to its distinct clinical and biologic features (Swerdlow et al., 2016). The clinical management regimen and utilization of radiation therapy of PMBCL varies amongst different

centers as there is no standard process. Even though a variation of chemotherapy methods in adult and pediatric groups have been studied, no consensus was reached on the optimal regimen of treatment (Giulino-Roth, 2018). A rituximab and anthracycline–containing regimen is used as the initial treatment at most centers. In United States the CHOP regimen (cyclophosphamide, doxorubicin, vincristine, prednisone) followed by subsequent R-CHOP (rituximab-CHOP) have been used as the standard for treatment of PMBCL (Kirn et al., 1993; Rodriguez et al., 2017). However, in some European centers, the more dose-dense V/MACOP-B (etoposide or methotrexate, doxorubicin, cyclophosphamide, vincristine, prednisone, bleomycin) regimens are used (Bertini et al., 2017; Klimo & Connors, 1985; Zinzani et al., 1996). For the majority of patients, CHOP and V/MACOP-B are usually dispensed in conjunction with consolidative radiation. Retrospective studies that were done in the pre-rituximab era hints that outcomes with V/MACOP-B are superior to CHOP (Todeschini et al., 2004) but the adjunction of rituximab to CHOP may lessen this difference between the two regimens (Avigdor et al., 2014; Savage et al., 2006).

Relapsed or refractory PMBCL normally ensues with a median time to progression of 8 months from diagnosis and a large number of relapse cases happen either during the therapy or within 12 months of conclusion of the therapy (Aoki et al., 2015; Kuruvilla et al., 2008). During the period of relapse, the disease can metastasize beyond the mediastinum, including the liver, pancreas, kidney, and the central nervous system. In case of patients with no history of receiving RT and whose disease is contained to the mediastinum, RT alone can be remedial (Dunleavy et al., 2013; Giulino-Roth et al., 2017). For all other patients, a treatment regimen of high-dose chemotherapy in conjunction with or in absence of RT, following autologous stem cell transplant (auto-SCT) is employed. Second-line treatment regime are comparable to those employed in case of DLBCL and include treatments such as R-ICE, R-

DHAP (rituximab, dexamethasone, high-dose cytarabine, cisplatin), and others (Giulino-Roth, 2018; Velasquez et al., 1988).

Chronic lymphocytic leukemia treatment

CLL diagnosis does not mean that the patients need to be treated. There are few indications that validate CLL treatment, counting constitutional symptoms, bulky lymphadenopathy, and splenomegaly that cause compressive problems; though, prognostic variables are important tools in a deciding a patient's therapy (Hallek, Shanafelt, & Eichhorst, 2018).

Currently, CLL does not have curative therapy. This results in palliation of symptoms and continuation of allowing to prolong survival are sensible therapeutic targets in case of majority of patients with CLL who need therapy as the disease has a potentially indolent course and most of the patients are elderly. Even so, experimental strategies to cure are merited in young patients with poor-risk factors. Current treatment options are discussed below based on prognostic factors and other variables (Hallek et al., 2018).

Early and stable disease

If symptoms progress or disease develops, people suffering from early, stable CLL do not need to be treated. Two types of evidence support this strategy. First of them is the fact that people with early and stable ailment can live to be same aged as disease free subjects (Montserrat, Viñolas, Reverter, & Rozman, 1988). Second, medical management with chlorambucil at an early stage (Binet stage A or Rai stage 0) either constantly or sporadically slows down the progression of disease but does not enhance survival chances. In addition, continuous chlorambucil treatment was associated with shorter survival due to the high incidence of epithelial cancers in one study ("Effects of chlorambucil and therapeutic decision in initial forms of chronic lymphocytic leukemia (stage A): results of a randomized

clinical trial on 612 patients. The French Cooperative Group on Chronic Lymphocytic Leukemia," 1990).

Advanced CLL with a large tumor burden and bone marrow failure

The standard treatment should still be considered to be chlorambucil given in the dose of 6 to 8 mg through the oral route every day; 0.4 to 0.8 mg orally every two weeks per kilogram of body weight. Therapy is administered till the patient improves, typically for 8 to 12 months or more. Rate of response vary between 40 and 70 %, but entire cure is rarely observed. The combination of chlorambucil and prednisone in recent trials do not show better compared to chlorambucil therapy alone. Although there are higher response rates for peoples treated with combination chemotherapy schedules, survival is not prolonged (Montserrat & Rozman, 1995) .

Cytopenia due to an immune mechanism or hypersplenism

A corticosteroid is the method of choice to treat people with cytopenia as to an immune mechanism, in combination to agents who were cytotoxic, given if the patient does not respond after four to six weeks of therapy. Other therapy options comprise of elevated-doses of immune globulin, splenectomy, cyclosporine, and low-dose spleen radiation (Montserrat & Rozman, 1995). In cases of hypersplenism, the latter two approaches may also be useful. CLL can rarely be associated with pure red-cell aplasia. Excellent results after treatment with cyclosporine have been reported (Chikkappa et al., 1992; Montserrat & Rozman, 1995).

Treatment of systemic complications

Hypogammaglobulinemia is a common cause of infection in CLL (Molica, 1994). In randomized experiment, immune globulin taken intravenously (four hundred milligram per kg in three weeks interval) stopped infection (Cooperative Group for the Study of Immunoglobulin in Chronic Lymphocytic Leukemia et al., 2010) but did not affect survival.

Cost-benefit concerns make it hard for all patients with hypogammaglobulinemia to routinely use immune globulin. Lower immune globulin doses can be as effective when compared to high doses (Kerr et al., 2014). Infections must be treated with antibiotics which were broad spectrum, and the chances of opportunistic infection infiltrating must also be considered when deciding treatment option. As the immune system is impaired, vaccines are thought to cause a response which was suboptimal (Cooperative Group for the Study of Immunoglobulin in Chronic Lymphocytic Leukemia et al., 2010).Treatment-related neutropenia can be overcome by recombinant hematopoietic growth factors such as granulocyte – macrophage colony-stimulating factor and granulocyte-stimulating factor (Rozman & Montserrat, 1995). Lastly, erythropoietin can be of use for treating anemia which does not respond to other mode of treatments (Pangalis, Poziopoulos, Angelopoulou, Siakantaris, & Panayiotidis, 1995).

Mantle Cell Lymphoma treatment

In many B-cell lymphomas, like FL or diffuse B-cell lymphoma, rituximab strengthened patient's survival rate and shifted the therapy strategy, decreasing the part of more aggressive front-line treatment. In MCL, on the other hand, patient perspective has still not changed pointedly, and PR actioners continue to face a fast spreading disease with a typically bleak forecast, an average 5-year survival, and a propensity to show early relapse and show inadequate response to salvage therapy. This ailment frequently includes the bone marrow, so collecting blood stem cells with the minimum quantity of contaminating tumor should be done as fast as possible if autologous transplantation is planned. For these factors, PR actioners use an active approach for young (< 60-65 years of age) and fit (no relevant comorbidity) patients in these cases, the clinical analysis of the cancer (whether mainly involving the lymph nodes, bone marrow, and spleen or intestine) is not relevant to the treatment selection. PR actioners in the institution decided to use 4 to 6 cycles of R-hyper-

CVAD / R-HD-MTX-Ara-C followed by a consolidation with BEAM and PBSCT. Although this approach has not been confirmed as ideal in randomized controlled trials, it is hypothesized to be safe and active when applied in tertiary centers by sufficiently expert staff and facilities in case of high dose therapy. As the occurrence of MCL patients younger than 65 years of age is similar to the incidence of AML cases of the same age, PR actioners believe that the option to treat them all in tertiary centers with expertise in acute leukemia treatment can be deemed accurate.

Patients who are unable to tolerate aggressive treatment, either due to age or comorbidity, are treated with reduced intensity palliative chemotherapy, usually with single agents. practitioners often still choose to give oral chlorambucil due to the favorable toxicity and cost profile of all possible options, eventually combined with rituximab. This combination therapy's response rate and duration is satisfactory and it has minimal impact on quality of life (Bauwens et al., 2005a) .

It is suggested survival improvement seen over last decade is not because of improved first-line therapy, but because of enhanced second-, third- and fourth-line treatment availability (Ghielmini & Zucca, 2009). Whether it is accurate or not, the target of the regimen becomes palliation of symptoms when patients relapse after an aggressive approach. It is fairly rare to see patients who are young and motivated and have a donor who is compatible is an exception to the treatment procedure: here clinicians take into account the chances of allogeneic transplantation and discuss the benefits and loss of undertaking such procedures.

In case of a chance of an allogenic translation, cisplatin-based regimen is induced like in case of other lymphomas.

During relapse, a variation of medications showed a good therapeutic index. These therapies consists of single agents as thalidomide, (Kaufmann et al., 2004) chlorambucil, (Bauwens et

al., 2005b) bendamustine, (Ghielmini & Zucca, 2009; Rummel et al., 2005) and cladribine, (Inwards et al., 2008) or more developed drugs such as bortezomib, lenalidomide, or temsirolimus (Ghielmini & Zucca, 2009). Combination treatments, such as R-FC (D. W. Thomas et al., 2005), R-FCM (Forstpointner et al., 2006) and gemcitabine/dexamethasone plus or minus cisplatin, (Morschhauser et al., 2007) and they produce a better response rate in most cases. However, they have no probable effect survival chances and have the chance of suffering from different side effects.

Finally, radiotherapy could be a good option in selected patients both localization irradiation or in the form of radiation immunotherapy. An issue with this latter form of therapy is that it involves less than 25 percent penetration of the bone marrow and a normal number of platelets, both of these settings are often not fulfilled in relapsed MCL (Ghielmini & Zucca, 2009).

Gastric MALT lymphoma

Physicians recommend that antibiotics be used as primary therapy for people with early-stage gastric MALT lymphoma. Careful monitoring of endoscopy and biopsy is required to monitor lymphoma regression. No clear guidance for treating relapsed or Pylori-negative disease exists. A crucial to note point is that a few patients that have recurrent disease can be treated by not using active therapy, and with an intact stomach they can continue to live a normal life (Cohen et al., 2006).

In an effort to determine the patients who would react positively to antibiotic therapy, Kuok et al. (Kuo et al., 2005) retrospectively examined CD86 expression (costimulatory molecule B7.2) and CD56 + natural killer cell infiltration in cancer cells amongst a group of patients with high-grade gastrointestinal MALT lymphomas. It was found that both factors correlated with H. Pylori dependency and direct therapy may be useful in spite of this, in people with

high-grade gastric MALT lymphoma that are not suitable candidates for chemotherapy, a clinician should always be hesitant to just use antibiotic treatment on its own.

Chapter 3

CAR T-cell therapy

The existence of naturally occurring tumor-reactive T cells in the peripheral blood and tumors of cancer patients has been thoroughly documented (Baitsch et al., 2011; Cohen et al., 2015; Fourcade et al., 2010; Hall et al., 2016; Junker et al., 2012). While T cells can recognize neoplastic cells, though, their numbers are often inadequate to mediate clinical regression of tumors. Tumors use a multitude of methods to counteract immune attack, especially T-cell-mediated responses (Beatty & Gladney, 2015; Croci et al., 2007; Rabinovich, Gabrilovich, & Sotomayor, 2007). These mechanisms, among others, include downregulation of the expression of MHC molecules, or disruption of antigen processing and presentation machinery (Garrido et al., 1997; Leone et al., 2013; Seliger et al., 2001). Chimeric antigen receptor (CAR) T cell therapy is a gene therapy based on which has been used in several phase I and phase II clinical trials to treat patients with relapsed or refractory malignancies. This individualized patient therapy utilizes the body's T lymphocytes ' natural function. Patient (autologous) or donor (allogeneic) T cells are modified to generate CAR T cells. The CAR is a chimeric construct that contains at least one signaling domain of T cell receptor and a single chain variable (scFv) fragment. T cells from a donor or from the recipient are genetically modified to express a particular CAR (e.g. using a retrovirus) engineered to recognize a specific tumor-related antigen; this allows T cells to actively target and selectively kill cells that express that antigen (e.g. CD19, HER-2). In patients with late-stage disease, early clinical trials investigating anti-CD19 CAR-T cells in hematologic malignancies have shown continued remission. In non-hematopoietic malignancies studies, CART cell therapy investigating various cancer-specific targets also showed some potential (Davila et al., 2014; Gross, Waks, & Eshhar, 1989; Jackson, Rafiq, & Brentjens, 2016; Lee et

al., 2015; Maude & Barrett, 2016; Porter, Levine, Kalos, Bagg, & June, 2011; Yu et al., 2017).

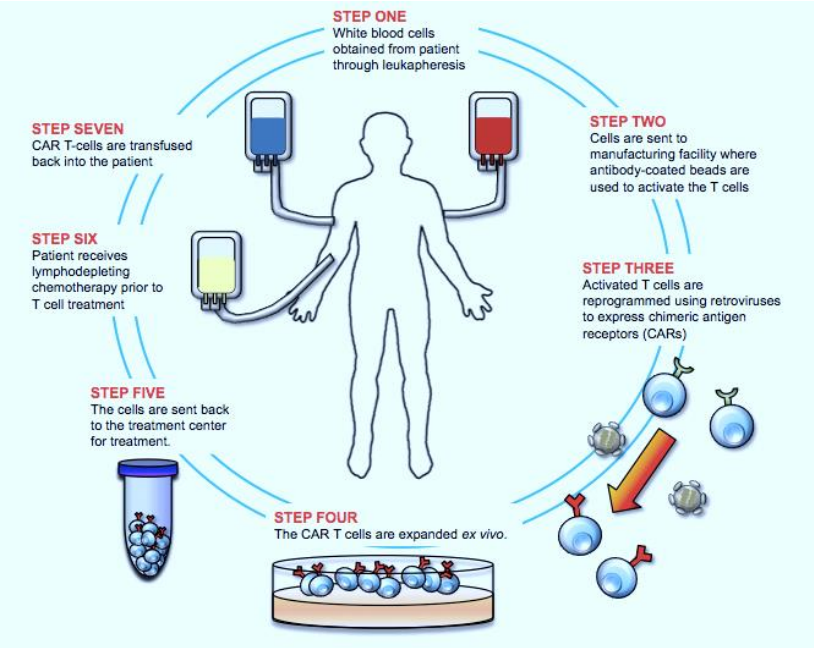

Figure 7-Car T-cell therapy (King, 2017)

3.1 Structure and mechanism

CAR T-cell therapy recently received acclaim due to its successful clinical trials and FDA approval (Maude et al., 2018; Neelapu et al., 2017; Schuster et al., 2017; Swann & Smyth, 2007). Chimeric antigen receptor T-cells express CARs on their cell surface. CARs are receptors located on the surface which have been modified and provide selectivity to T-cells against a pre-identified target antigen, which is being expressed on tumor cells.

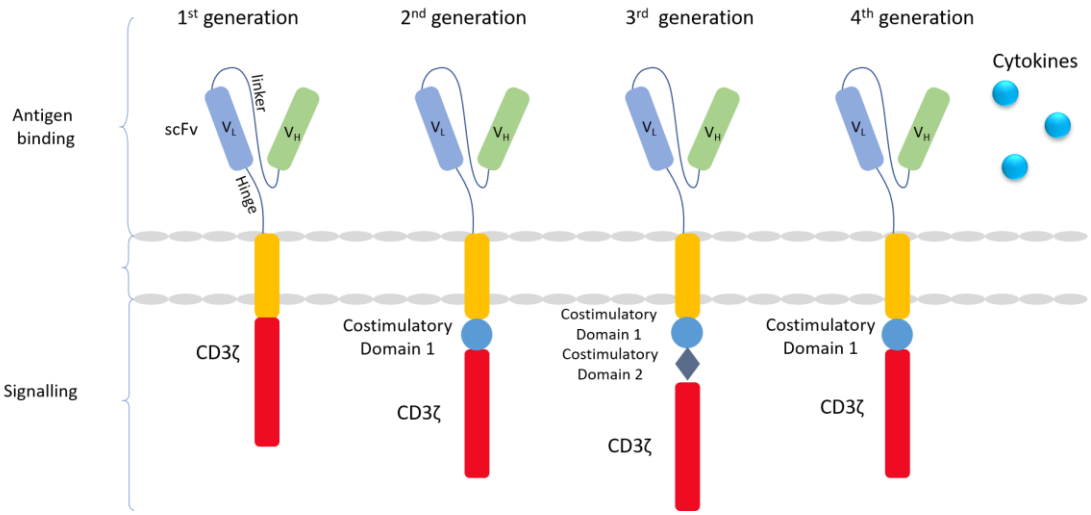

Figure 8-Chimeric antigen receptor (King, 2017)

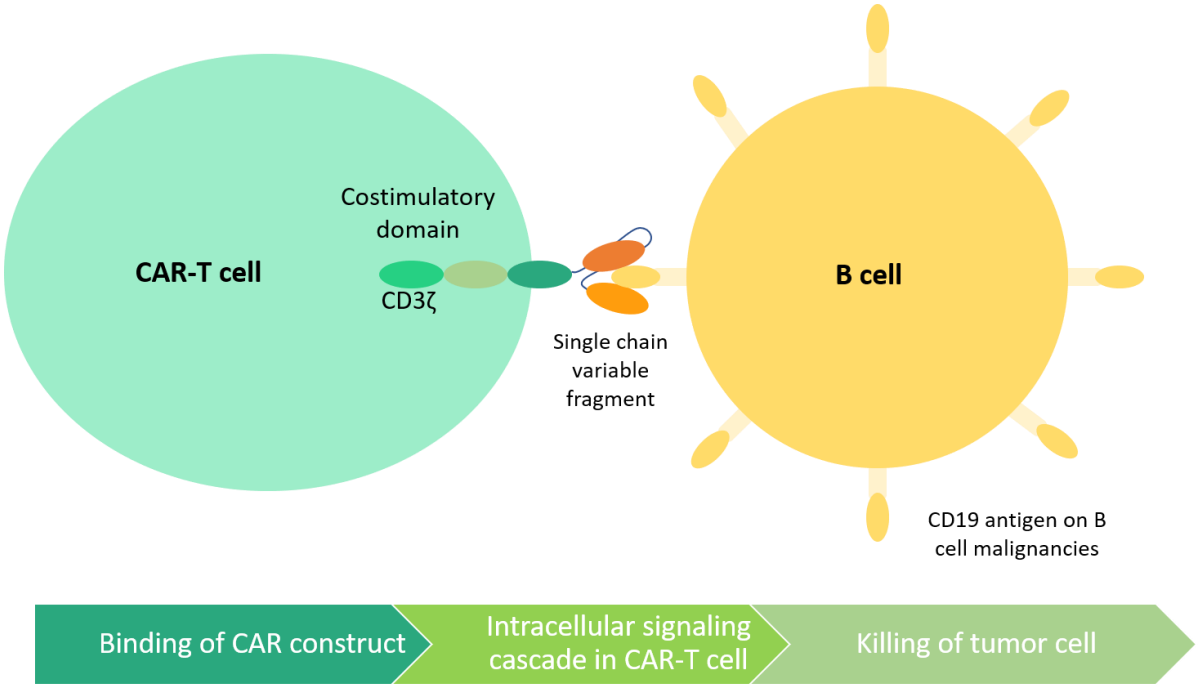

Figure 9-Schematic diagram of CAR T-cells (Graham, Hewitson, Pagliuca, & Benjamin, 2018)

The T-cell receptor is composed of antigen-specific α and β chains connected to the CD3 complex (ε, δ, γ and ζ chains). It is used to recognize the target antigen presented in conjunction with the MHC. Alternatively, the CAR has an extracellular domain which consists of a single-chain variable fragment (scFv) that recognizes a specific tumor antigen and is linked to a transmembrane domain that is linked to the CD3 signaling unit and the CD28 or 4-1BB co-stimulative units. This complex is the domain of the intracellular (June &

Sadelain, 2018; Salter, Pont, & Riddell, 2018). CAR T-cells have the ability to identify antigen-independent specific tumors of the MHC complex. The first generation of CARs were encompassed of only CD3 as a signaling molecule and although the T-cells expressing these CARs killed the target T-cells, they did not easily proliferate and failed clinical studies (Brocker, 2000; Jensen et al., 2010). The innovation of second and third generation CARs has resolved this problem. These have an intracellular domain that consists of CD28 or 4-1BB costimulatory molecules linked to CD3 in tandem. The signal to activate is supplied by the CD3 SUB. The costimulatory molecules increase the production, proliferation and survival ability of cytokines (Krause et al., 2002).

The signal to activate is provided by the CD3 SUB. The costimulatory molecules increase the production, proliferation and survival ability of cytokines (Jensen et al., 2010). To overcome the inhibitory tumor microenvironment, particularly in solid tumors, the 4th generation "Armored CAR T-cells" has now been developed. In addition to the typical CAR functionality, armored CARs are able to secrete cytokines such as IL-2, IL-12 transcribed from an independent gene incorporated and transduced by the CAR vector (Pegram, Park, & Brentjens, 2014; Yeku & Brentjens, 2016).

3.2 Design of CAR T-cell therapy

CAR T-cell therapy relies upon effective, steady and secure platforms for transferring genes. Autologous T-cells are produced and modified genetically by isolating leukapheresis, using methods of viral and non-viral transfection ex vivo. Culture is used as means of expansion of modified T-cells. Following infusion of CAR T-cell, the patient is treated with lymphodepleting chemotherapy in most cases in case of the CAR T-cell products that pass the quality control tests. The CAR's extracellular domain consists of two things - the moiety which binds with the antigen and a spacer. Such moieties that bind to antigen may be of three

types: a) a scFv; b) a human Fab fragment or c) nature ligands (Sadelain, Brentjens, & Rivière, 2013). scFv, which is expressed on the tumor's surface cell, is a variable antibody fragment which is monoclonal in nature and can be derived from humanized Abs, mouse monoclonal antibodies (mAbs) or fully human Abs and can identify and bind with tumor-associated antigens (TAAs). In contrast to normal TCRs, CARs recognize unprocessed antigens, glycolipid and carbohydrate structures and are generally expressed on the surface of tumor cells without presence of MHC antigen (Schmidt-Wolf, Negrin, Kiem, Blume, & Weissman, 1991). By bypassing the limitation of MHC class I and class II, CAR T-cells can be recruited from subsets of both CD4 + and CD8 + for recognition of redirected target T-cell. The CAR-mediated elimination mechanism of tumors by redirected CD8 + and CD4 + T-cells mainly uses two pathways in the execution of cytolysis, e.g. granzyme and perforin exocytosis in some extent, the signaling of the death receptor via TNF / TNF-receptor (TNF-R) or Fas / Fas-ligand (Fas-L) (Chmielewski, Hombach, & Abken, 2013). IgG1's hinge region is the simplest spacer form and is sufficient for maximum constructs founded on scFv (C. Zhang, Liu, Zhong, & Zhang, 2017). The linking amid the antigen domain which is accountable for binding and the transmembrane domain (TM) is called a spacer. The steadiest of the receptors is called CD28 TM.CD3, the usual component of the intracellular domain, enables the activation and function of T-cell to deliver the first signal. For enhanced cytokine secretion (IL-2), T-cell expansion and persistence concomitant co-stimulatory signals (CD28 or 4-1BB) are secondary signals required in vivo (Singh, Frey, Grupp, & Maude, 2016; C. Zhang et al., 2017). The domain of intracellular signals was widely researched both preclinically and clinically and can have a significant impact on CARs functional activity (Priceman, Forman, & Brown, 2015). There are 4 generations of CAR T-cells depending on the composition of the intracellular domain (C. Zhang et al., 2017). First-generation CARs consisting of TCR / CD3 (CD3) complex chain. CARs of second generation are

characterized by a dual T-cell activation signal: antigen recognition triggers one and costimulatory fragment such as CD28/B7 produces the other one, which also promotes synthesis of IL-2 to finish activating T-cells and circumvent apoptosis (C. Zhang et al., 2017). CARs of the third generation enhanced response by merging sequences of co-stimulatory signals such as CD3,DAP10 ,4-1BB (CD137), OX40 (CD134), CD28 or CD27 or other CD3 molecule (Carpenito et al., 2009; Zhong, Matsushita, Plotkin, Riviere, & Sadelain, 2010). Through increased cytokine production, proliferation of T-cell and killing in recurrent antigen exposure the combination of multiple co-stimulatory signals can increase CAR T-cells function (Priceman et al., 2015). More researches are needed to investigate the level of safety and effectiveness of CARs of the third generation (Sadelain et al., 2013). Redirecting CAR T-cells for universal cytokine killing (TRUCK) can be a method for further optimization of CAR's design as suggested by many reports. TRUCK cells produce a transgenic product such as IL-12 or IFN-π and release it afterwards (Yu et al., 2017). IL-12 has the ability to activate immune reactions which are innate to tumor cell that are invisible to CAR T-cells. IFN-π, expressed in tumor stroma, can play a role in antigen-independent tumor cell destruction through IFN-πR (Curran et al., 2015; Yu et al., 2017). To provide synergistic killing and improved function, a biphasic CAR (tandem CAR-TanCAR) consisting of a single transgenic receptor that can recognize two antigens with distinct properties is designed. A flexible hinge separates the identification domains for the two distinct antigens in tandem. This approach bypasses loss of antigen and tumor escapement; TanCAR remains functional and maintains T-cell cytolytic capability even if one of the antigens that are targeted is de-regulated or mutated (Grada et al., 2013). Wilkie, et al. and Kloss, et al. have hypothesized dual specific CARs to achieve improve tumor specify. The proposal suggests that two distinct CARs are co-expressed in the same T-cell population and can improve tumor specificity by each being able to recognize a dissimilar antigen of tumor and providing additional cues.

Only tumors which are positive for double antigens are killed in this strategy known as **"tumor barcoding".** These CAR T-cells have included a CAR which offers inadequate CD3-mediated activation when one antigen and a co-stimulatory chimeric receptor is binding. In these cases, the cp stimulatory chimeric receptor comprises of only CD28 and 4-1BB which acknowledges a second antigen. By providing CAR T-cell with specific target site binding and preventing off-target bindings, it ensures full activation of T-cell once it fulfills the objectives of the two CARs (Kloss, Condomines, Cartellieri, Bachmann, & Sadelain, 2013; Wilkie et al., 2012). Two "universal" CAR systems were reported in addition to antigen specific approaches. These systems comprise of CARs that contain scFv for avidin (Urbanska et al., 2012) or isothiocyanate antifluorescein (FITC) (Tamada et al., 2012) for tumor identification which are related with, biotinylated or FITC-bound, mAbs. By employing an antigen-specific CAR (iCAR) inhibitor incorporated in T-cells, T-cells recognizing antigen both in tumor or off-target tissues could be limited to only tumor cell and thus off target tissues are protected. Dynamic, self-regulating safety switch is provided by iCARs to stop the effects of suboptimal T-cell specificity rather than treating them. These T-cells contain an additional CAR, iCAR, that targets distinct, off-target tissue antigen coupled with a powerful, inhibitory PD-1 or CTLA-4 intracellular signaling domain**.** After interaction with the antigen of off-target tissue, these cells can chose to selectively limit secretion of cytokine ,tendency to cause cytotoxicity and proliferation (Dai, Wang, Lu, & Han, 2016; Fedorov, Themeli, & Sadelain, 2013; Juneja et al., 2017). Combination strategies that combine CAR T-cell therapy and inhibitor checkpoint blocking, using antibodies which are antagonistic in nature in response to CTLA-4 and PD-1/PD1-L negative regulators how great potential. The specific blockage of the PD-1 immunosuppressive pathway has been shown to significantly enhance the activity of anti-HER2 CAR T-cells which led to tumor eradication in immune-competent HER2 transgenic mice (John et al., 2013).

3.3 Genetic engineering

Since 1970, methods of genomic engineering have been advanced from base level physical-chemical research laboratory techniques to methods of viral and non-viral transfection, with an aim to obtain high transgenic expression with lesser amount of oncogenic or harmful negative effects. This study explains the rudimentary structure of CAR-engineered cells with multiple methods that can be applied in clinical practice to transfer gene, counting methods of transposons, viral transduction, mRNA transfection and RISPR / Cas9 technology, electroporation, nanoparticles, liposomes. This review also discusses the advantages and disadvantages, of the therapy highlighting long-lasting transgenic expression with lesser concerns of safety.

3.3.1 Viral Transduction

Viral transductions process is right now hailed as a favored method employed in outfitting T-cells with CARs. Vectors which are viral in nature and belong to the "Retroviridae" family are generally utilized vectors in gene therapy procedures presently. Significant advantageous circumstances obtained by using gene transfer vectors include the overall simplicity of manufacturing and production and ability to steadily incorporate the genetic material with the genome of host. So as to conform to norms of clinical security, viral vector platform has to show inability to replicate, low level of genotoxicity and low level of immunogenicity. Mainly two characterizing qualities of retrovirus species gives them the ability to be especially fit to work as vectors used in gene transfer: (I) the vast majority of the viral genome can be supplanted with transgenes of interest; (ii) ensuing transduction, the genome is incorporated into the genome of host cell permanently. Therefore, simple γ-retroviruses, were the first designed packaging systems for gene transfer which was effective. Lentiviral vectors which were most regularly utilized depend on the human immunodeficiency infection

(HIV). To produce a CAR vector, the required genes gag, pol and env (plus rev for lentivirus) are taken out from the viral spine and they are given in trans, in helper plasmids, for viral generation. CAR transgene, not viral genes, is introduced. Activated T-cells, carrying OKT3/CD28 globules, are incubated with retroviral particles for genomic inclusion. After the combination of host and viral membrane, the core which is virion is discharged in cytosol and transported in line with the microtubules to arrive at the nucleus. This process permits expressing high levels of CAR while producing T-cells (Piscopo et al., 2018; Tumaini et al., 2013). The long terminal repeats (LTRs) are known as viral control center for expression of gene which act as polyadenylation signal, enhancer, promoter, t transcription terminator, transcription initiation (capping). Albeit, 3′LTR in addition 5′LTR have a similar sequence, The security levels of these vectors are high these days, coming about because of the partway removal of the U3 locale of 3′LTR and the utilization of cytomegalovirus (CMV) promoter to supplant the U3 area at 5′LTR to begin the transcription process. The procedure decreases transcriptional action from LTR's infection. Be that as it may, there is as yet a danger of insertional oncogenesis at arbitrary locales inside the genome and a possibility of immune mediated toxicity, brought about by extended persistence and movement of designed T-cells. Additionally, there are confinements of size and number of genes which can be stuffed in the vectors and the problem of stable efficacy. What's more, heterogeneous duplicate statistics result in T-cell populaces with profoundly cytotoxic variable capacities, because of various amounts of cell surface expression. There are extra assembling matters identified with viral carrier, that have high cost of production. Whereas the size of manufacture for viral production was adequate for Phase I/II clinical trials, financially effective application of virum mediated CAR treatment remains a major barrier (Androulla & Lefkothea, 2018).

3.3.2 Transposons

Transposons are double genetic components which have mobility and made out of: (a) one plasmid with the CAR transposon and (b) another transposase resonant plasmid. The bicomponent vector frameworks, for example, Sleeping Beauty (Jin et al., 2011) and piggyback (Manuri et al., 2009), can prompt the steady integration of a transgene. There is a fundamental mechanism to the frameworks incorporates the transposase, that act on inverted terminal repeats (ITRs)) flanking the CAR succession, in this way prompting excision and consequent combination at a TA dinucleotide inside the genome of target cell. Plasmids inside the DNA which carry the CAR (as the transposon) and transposase are electroporated into T-cells. After transposition and genomic integration which is stable in nature, the CAR protein expresses itself on surface of T cell. Transposon-mediated CAR treatment shows significant efficacy shows lower toxicity levels, with decreased production expense in addition to increased speed of production contrasted with the traditional plasmids, when transfected into mammalian cells. Contrasted with Sleeping Beauty, piggyback framework appears to have an advanced quality exchange proficiency, short of reintegration near proto-oncogenes, and creates CAR T-cells, despite the fact that it has not be clinically tried (Oldham & Medin, 2017).

3.3.3 CRISPR/Cas9

During start of 2000, the scientific groups turned their attention into genetic “editing”. Zinc finger (ZFNs) and nucleoside translation activator-like effector nucleases (TALENs) were created. ZFNs and TALENs are chimeric, custom fitted restriction proteins(enzyme) which are designed to be used against explicit genetic sites, including approved as safe harbor sites. Up until this point, in CAR treatment, the innovation has continued to be utilized to knock out the endogenous TCR receptor in allogenic T cells, that has the ability to prevent

undesirable graft versus-host disease (GvHD), in spite of this fact that the CAR transgene was virally transfected (Cellectis - UCART19) ("Preliminary Data from Servier and Pfizer's UCART19 Product Candidate Shows High Complete Remission Rate Across Two Phase I Adult and Pediatric Acute Lymphoblastic Leukemia Trials | Cellectis," 2017; Qasim et al., 2017). It is possible to hinder or postpone the refusal of CAR T-cells by host's immune system by employing genome-editing tactics and reducing or even eliminating the histocompatibility antigen's expression on donor T-cells. CRISPR/Cas9 was a revolution in genetic editing technology. A short RNA guide (gRNA) can direct the type II CRISPR protein Cas9 for targeting regions inside the genome, where it acts as an endonuclease. The endonuclease may be transferred to reach its Cas9 protein/ gRNA ribonucleoprotein (RNP) (Liang et al., 2015) or as a plasmid, driven by either U6 or H1 promoters for transcription after transfection of mammalian cells. The transfer can be done in many ways such as, electroporation, liposome mediated transfection, as part of a viral genome or chemical transduction (DeWitt, Corn, & Carroll, 2017). Following that, the selected transgene is integrated by homology-directed repair (HDR), using a donor template, which generally is in a plasmid form. Moreover, a substitute non-viral method is implemented via nanomaterials. One of these methods increases the speed of transfer of genes up to five times than seen in typical means. At last, co-injection of Cas9 (delivered as mRNA transcribed in vitro (IVT-mRNA) with a solitary gRNA species improved genomic cleavage speed in case of some cells (Wang et al., 2013). CRISPR technology was used in the production of CAR T-cells which shows to have a comparatively higher degree of homogeneity and has shown promise in the survival results of mouse model. In particular, the inclusion of the CAR sequence in to the endogenous T-cell receptor locus "alpha constant "- TRAC decreased the CAR T-cell cytotoxicity (MacLeod et al., 2017). Furthermore, the effectiveness of gene editing for CAR knocking has shown to be little, with rate of success of it being up to 20 percent

.Additionally it has the problem of off target mutagenesis persists (Cyranoski, 2016). CRISPR / Cas9 knocked out PD-1 and endogenous TCR in T-cells lung cancer patients in the first CRISPR / Cas9 clinical trial. Though, in this trial, TCR or CAR were not incorporated` into T-cells. Similar studies are also being initiated with autologous renal cell carcinoma (NCT02867332), prostate T-cells (NCT02867345) and bladder cancer (NCT02863913). The main objective is to eliminate the chance incorporation of viral systems of delivery and to regulate the integration of CAR. Whether removing certain signals known to be inhibitory from the T-cells results in unregulated cell proliferation or severe autoimmunity is unclear (Ren & Zhao, 2017).

3.3.4 Non-viral Transfer Methods

The transfer of TCR genes to primary T-cells through electroporation of mRNA was discussed by Zhao et al (Yu et al., 2017). The construction of a therapeutic regimen using mRNA addressed multiple issues and appeared as a key challenge due to its features like sensitivity and vulnerability to degradation, unsteadiness, negative load, inadequate translation in host cell and effects related to immunoregulation. To some extent, the challenges were avoided by a better view of the association amid mRNA structure and stability and the growth of a wide range of methods for chemical modification. Adding anti-reverse cap analogs (ARCAs) and polyadenylate tail are the various structural modifications of mRNA. These changes increase translation efficiency and mRNA stability. The tail of poly (A) is favored to exceed hundred residues. Additional alteration is the substitution of components of adenylate- uridylate rice (AREs) with increasingly stable elements of the β-globin gene 5'UTR (untranslated region) and 3'UTR. The extensively studied AREs are significant signs of mRNA decay in most eukaryotic mRNAs' 3'UTRs. MRNAs comprising AREs signify reduced stability, possibly due to poly (A) tail elimination. Stability shows improvement but, when AREs are substituted by the 3'UTR of a more stable mRNA, such as

β-globin mRNA. These structural changes increase the stability of mRNA and allow for extended periods of expression. The transfer of mRNA is a system of cytoplasmic expression; entry to nucleus is not needed to arbitrate its function. IVT- mRNA can be produced with changes to its structure that enhances products stability. Therefore, it is necessary to further improve the modes of delivery of mRNA so that it can advance as a therapeutic tool. IVT-mRNA delivery may be facilitated either by cell membrane disruption (electroporation, gene gun) or by endocytosis through the use of several nanoparticles (Moffett et al., 2017). Lipofectamine is widely used to introduce IVT-mRNA into cells as a cationic carrier. Lipofectamine consists of cationic lipids forming liposomes with surfaces that have positive charge and facilitating the mRNA's entry inside the eukaryotic cell through endocytosis as follows: positively charged liposomes crosslink with nucleic acid backbone phosphate groups and make a complex that causes a reaction with the cytoplasmic membrane which is negatively charged, thus allowing the formed complex to merge therewith. The moiety builds up intracellularly, manages to evade from the endosome and enters the cytoplasm to be expressed by the genetic material (Cardarelli et al., 2016). Among the most successful methods for presenting the CAR IVT-mRNA construction into T-cells is electroporation. It has come to notice that IVT-mRNA transfection via electroporation was sufficiently effective under certain circumstances significantly lower number of apoptosis linked to the electroporation (Zhao et al., 2006). The transfection systems mediated by mRNA enable faster variations in CAR design and comparatively safe in the long run, integrated, viral expression systems. Utilization of IVT-mRNA transfection methods provides additional safety for CAR therapy and the clinical advantage required is obtained despite short lifetime and expression transiency. Indeed, degradation of IVT-mRNA over periods of time ensures the patient's entire elimination of the CAR without the necessity for suicide genes (Angel & Yanik, 2010). As a result, IVT-mRNA-mediated transfection systems are more easier to

transfer in a system that complies with GMP with possibly decreased costs and less complicated release tests (Barrett et al., 2013). In reality, only a few repetitive CAR T-cells infusions (3-9 infusions) are needed in case of patients to elevate a enduring response (G. L. Beatty et al., 2014). At the University of Pennsylvania (Philadelphia, PA; NCT02624258, NCT01837602, NCT02277522, NCT02623582), CARs transfected to T-cells using mRNA are presently under investigation. Furthermore, new regimens to deal with solid tumors using CAR T-cells modified by IVT-mRNA which were electroporated (Brown et al., 2015). Though, electroporation occasionally leads to cell death in a process known as irreversible electroporation, especially if electrical fields induce permanent permeation of membrane and the resulting in the loss of cell homeostasis. Using surface plate electrodes, when the electroporation field is applied to the skin, the main "potential" drop develops in line with the skin instead of being along the target subcutaneous tissues. Inflammation of the skin is a normal outcome of electroporation in vivo. Maximum number of protocols of electroporation are intended to enter only the plasma membrane. The electroporation of nucleus requires a further step which is using increased voltage and lower pulse strength known as nucleoporation. Moreover, while the principle of electroporation applies to all types of cells, its efficacy relies on the cells ' electrical properties. Smaller cells need to penetrate a higher field in size. Cells which contain less conductive contents are less sensitive (such as fat cells). Thresholds will thus vary for diverse cells in a varied tissue. The utilization of IVT-mRNA instead of MC- or plasmid-encoded transposase and CAR gene is desirable because of elimination of intentional integration into the host genome. The primary disadvantages of such systems, however, are the long ex vivo cultivation period to produce effective doses of gene-modified T-cells and increasing cell damage that can follow by the plasmid DNA electroporation.

3.4 FDA approved indications

There have been three recent approvals from the U.S FDA aimed to be used as anti-CD19 CAR T-cells in case of malignancies of B cell due to clinical trial success (Maude et al., 2018; Neelapu et al., 2017; Schuster et al., 2017). Tisagenlecleucel which is marketed as KYMRIAH has received approval in August 2017 in case of refractory or recurrent B-cell precursor for to be used in the age category of child and people who are of less than 25 years of age and recurrent or refractory (RR) DLBC) which do not have any special specifications in May 2018 ("KYMRIAH (tisagenlecleucel)," 2018). Tisagenlecleucel can be described as a CD19 directed 4-1BB/CD3ζ CAR T-cell treatment procedure. The process involves using autologous T-cells which are modified via genetic means so that it can be used to express anti-CD19 scFv linked to signaling domains 4-1BB and CD3ζ. In case of relapsed or refractory (R/R) DLBCL which have no notable features , primary mediastinal large B-cell lymphoma, high-grade B-cell lymphoma and DLBCL arising from follicular lymphoma Axicabtagene ciloleucel (YESCARTA), CD19 directed CD28/CD3ζ CAR T-cell therapy, received approval in October 2017 (US FDA, 2018).

3.5 Efficacy and safety

Three CAR T-cell based drugs which are characterized as second generation are currently being tested targeting CD19: axi-cel, tisagenlecleucel and lisocabtagene maraleucel (lio-cel). Liso-cel employs CD137 costimulatory domain and its difference with other drugs lie in the fact that the ratio of CD4:CD8 CAR T cells is fixed at 1:1. The drugs showed great results in early stage trials in academic institutions where they were developed and later were licensed to pharmaceutical companies. In the ZUMA-1 study, after conditioning chemotherapy of cyclophosphamide and fludarabine, axi-cel was administered on a target dose of 2×106 CAR T cells per kg of body weight on day 0. 108 patients were treated in total and among them the

observed best ORR had been eighty two percent and observed rate of CR stood at fifty eight percent. Of the total treated, forty two percent of the treated people continued to enjoy remission and the median time of follow up was observed to be 15.4 months. It was observed that around 5.8 months was the time period of median progression-free survival and overall survival was after a follow up period of 12 months was fifty none percent (Neelapu et al., 2017).

93 people were treated with Tisagenlecleucel and among then best observed ORR had been fifty two percent and observed CRR was at forty percent. Of the patients treated the overall survival statistics are shown to be forty nine percent after a 12 month follow up period. It was observed that 2.9 months was the median progression-free survival time. In the patients treated with CAR T-cell therapy Cytokine release syndrome (CRS) as well as neurotoxicity are observed to occur after the treatment. More rare complications include (HLH (MAS), anaphylaxis, and tumor lysis syndrome. Ablation of normal B cells causes prolonged B cell aplasia after being treated with anti-CD19 CAR T-cell therapy causing hypogammaglobulinemia. This phenomenon is widely seen and can be treated with immunoglobulin replacement therapy for patients that suffer from recurrent infections. In most cases all these events can be categorized as grade 1-2 and some are categorized as grade 3-4. All of these events are generally reversible. To manage these events a multidisciplinary approach is usually taken. The multidisciplinary approach involves close hemodynamic monitoring, aggressive medical and supportive care and use of specific drugs including tocilizumab, an anti-IL-6 receptor antibody, and/or corticosteroids (Neelapu et al., 2018).

3.6 Importance of conditioning therapy

Conditioning chemotherapy is given to patients prior to CAR T-cell therapy to increase its efficacy which is achieved by multiple mechanisms; it depletes normal lymphocytes to create

space for proliferated CAR T-cells (Klebanoff, Khong, Antony, Palmer, & Restifo, 2005), increases accessibility of certain cytokines to encourage proliferating tendencies of CAR T-cell (Gattinoni et al., 2005; Kochenderfer et al., 2017; Turtle et al., 2016), reduces immunosuppressive cells (Klebanoff et al., 2005; Wrzesinski et al., 2010).

Chapter 4

Conclusion

4.1 Conclusion

Cellular therapy and immunotherapy for cancer are now strongly at the forefront of anticancer treatment. Chimeric antigen receptor T-cell treatment remains a promising growth therapies for aggressive B-cell lymphomas. The high rate of success of CAR-T cell therapies has gained the attention of both the science community and society as a whole. This opens up a universe of opportunities for increasingly effective and decreasingly toxic cancer treatments. The route ahead of us, however, is not without difficulties. Two significant fronts will warrant particular attention as researchers work towards universalizing adoptive T-cell therapies beyond a few particular types of cancer: expanding clinical success to solid tumors; and improving tumor specificity to avoid toxicity associated with treatment. In both cases, a comprehensive and integrated understanding of the complicated signaling channels induced in cells, tumor cells and stromal cells are required. Certainly, the latest FDA approval of two anti-CD19 CAR T-cell treatment and long-lasting remissions found in recurrent or refractory aggressive B-cell lymphomas indicate that this could be the future. The therapies are now considered in earlier therapy lines and contrasted at first relapse in randomized research directly with high-dose chemotherapy and ASCT. Furthermore, their efficacy for both indolent B-cell lymphomas and mantle cell lymphoma is being assessed. With the intention to improve the safety and efficacy of these methods, combination strategies, bi- and multi-specific CAR T-cell therapies and tunable CARs are being developed.

4.2 Future direction

In spite of the increasing amount of interest sphere around CAR T – cell therapy, some challenges remain and further studies need to be done in those fields.

Hematological tumors are widely present in the circulation system. One promising strategy for treating patients with leukemia in current years involves the utilization of CAR T-cell therapy. Research is required to define new objectives and new combinations for hematological malignancies. CAR T-cell treatment is presently being studied in 420 clinical trials and possibly thousands of combinations. To define prospective combinations, the challenge will be to pick better preclinical models.

While CAR-T cell findings in B-cell malignancies show encouraging results, therapy with comparable methods for solid tumors has produced less favorable outcomes. Similar to hematologic malignancy treatments, the design challenge starts with building the CAR based on an antibody which is only expressed in tumors and not in ordinary tissue, for enhancing effectiveness whereas decreasing toxicity. Further research is required for solid tumors to define better target site or and to overcome barriers in the tumor microenvironment that block T-cell function.

Many cancer patients, owing to gained tumor resistance, do not react to immune control point treatment and some relapse. In cancer immunotherapy, epigenetic targeting can be useful by reversing immune prevention and escape mechanisms used by cancer cells and through modulating the differentiation as well as function of immune cells. More research is needed in these fields.

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